# YeastBuster™ Protein Extraction Reagent

## About the Kits

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| YeastBuster Protein Extraction Reagent | 100 ml | 71186-3 |
| | 500 ml | 71186-4 |

### Description

YeastBuster Protein Extraction Reagent is formulated for a fast, efficient and gentle extraction of active proteins from yeast cells. The proprietary formulation utilizes a mix of mild detergents and protein stabilization buffer that, when combined with tris(hydroxypropyl)phosphine (THP) reducing agent, effectively releases protein from yeast cells (1). This powerful combination eliminates inconsistencies associated with tedious mechanical disruption of yeast cells using glass bead abrasives, ultrasonication, pressure disruption, or enzymatic disruption with β-1,3-glucanase lytic enzymes. The reagent has been tested with *Saccharomyces cerevisiae*, *Pichia pastoris*, *P. stipidis*, *Schizosaccharomyces pombe* strains, as well as with filamentous fungi and plant cells (2-3).

YeastBuster Protein Extraction Reagent plus Benzonase® Nuclease is an efficient combination for gently releasing target proteins and markedly reducing extract viscosity prior to downstream processing. Yeast cells suspended in YeastBuster Reagent with Benzonase Nuclease are briefly incubated while soluble proteins are released and nucleic acids digested. Insoluble protein and cell debris are easily removed by centrifugation. The resulting low viscosity extract contains soluble proteins ready for additional purification or analysis. In addition to higher total protein yields in crude extracts and recovery of enzymatically active proteins, the extracts are fully compatible with GST•Bind™ and Ni-NTA His•Bind® immobilized metal affinity chromatography (IMAC) purification methods and reporter assays [e.g., GST•Tag™ Assay Kit (Cat. No. 70532-3), BetaRed™ β-Gal Assay Kit (Cat. No. 70978-3)]. For applications that require no reducing agent, YeastBuster will also lyse yeast cells in early log phase without the addition of THP; however, total protein extraction efficiency is decreased under these conditions



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**FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.**

## Components

- 100 or 500 ml YeastBuster™ Protein Extraction Reagent
- 1 or 5 × 1 ml 100X THP Solution

## Available Separately

- 25 KU Benzonase® Nuclease HC, Purity > 90% (Cat. No. 71205-3)
- 10 KU Benzonase Nuclease, Purity > 90%, (Cat. No. 70746-3)
- 2.5 KU Benzonase Nuclease, Purity > 90% (Cat. No. 70746-4)

## Storage

Store YeastBuster Protein Extraction Reagent at room temperature or 4°C. Store 100X THP Solution at –20°C.

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## General Considerations

- YeastBuster™ Protein Extraction Reagent is effective on yeast cells harvested from either log phase or stationary phase. However, stationary phase cells may require longer incubation times in the presence of YeastBuster plus THP due to the thicker cell wall of stationary phase cells.
- YeastBuster can be used on fresh or frozen cell pellets. For comparisons of multiple samples (e.g., extended time course analysis) all cell pellets should be processed identically (all fresh or all frozen).
- YeastBuster and Benzonase® Nuclease are most efficient when used at room temperature. Storage of YeastBuster at temperatures below 4°C may cause precipitation of the detergents. Incubate in room temperature water bath with gentle swirling to redissolve.
- YeastBuster Reagent is compatible with protease inhibitors and EDTA. Note that > 1 mM EDTA will interfere with protein binding to Ni-NTA His•Bind® Resin.
- Extracts prepared with YeastBuster are not compatible with protein assays sensitive to detergent or reducing agents. Therefore, we recommend the Non-Interfering Protein Assay™ Kit (Calbiochem Cat. No. 488250) for protein determination.
- The reporter assays GST•Tag™ Assay Kit (Cat. No. 70532-3) and BetaRed™ β-Gal Assay Kit (Cat. No. 70978-3) are compatible with YeastBuster Extracts.
- The ionic strength of YeastBuster extracts (250 mM) will prevent protein binding to ion exchange resins. Dialyze the extract against the desired ion exchange column-loading buffer prior to chromatography.
- Benzonase Nuclease can be diluted with 50 mM Tris-HCl, 20 mM NaCl, 2 mM $MgCl_2$, pH 8, for handling small quantities. This solution can be stored at 4°C for several days without loss of activity.

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## Extract Preparation

1. Harvest cells from liquid culture by centrifugation at 3,000 × g for 10 min at 4°C using a pre-weighed centrifuge tube. For small-scale extractions, (1.5 ml or less) centrifugation can be performed in a 1.5-ml tube at 5,000 × g for 3 min.
2. Decant supernatant and allow the pellet to drain, removing as much liquid as possible. Determine the wet weight of the pellet.
3. Resuspend the cell pellet in room temperature YeastBuster™ Reagent and THP Solution by pipetting or gentle vortexing. Use 5 ml YeastBuster Reagent and 50 µl 100X THP Solution per gram wet cell paste. For small cultures, use up to 1/30 culture volume for resuspension (e.g. use 50 µl YeastBuster for 1.5-ml cultures). There are no adverse effects to using higher ratios/volumes of YeastBuster.
   **Optional:**
   a) YeastBuster and 100X THP Solution can be premixed. This mixture is effective up to one month when stored at 4°C.
   b) Add 1 µl (25 U) Benzonase® Nuclease per 1 ml of YeastBuster. No addition of $Mg^{2+}$ is required for viscosity reduction and nucleic acid digestion under the conditions described here.
   c) Add protease inhibitors. Protease inhibitors are compatible with YeastBuster and Benzonase. Serine protease inhibitors should be avoided if the target protein is to be treated with Thrombin, Factor Xa or Recombinant Enterokinase. Avoid the presence of serine and cysteine protease inhibitors if the target protein is to be treated with HRV 3C. Although purification may remove active inhibitors, dialysis and gel filtration are recommended prior to cleavage.
4. Incubate the cell suspension on a shaking platform or rotating mixer at a slow setting for 15–20 min at room temperature. If Benzonase was added, the extract should not be viscous at the end of the incubation.

*Note: If using* S. pombe*, increase incubation temperature to 45°C.*

5. Remove insoluble cell debris by centrifugation at 16,000 × g for 20 min at 4°C.
6. Transfer supernatant to fresh tube. The soluble extract is ready for analysis or further processing. Maintain clarified extracts on ice for short term storage (2–3 h) or freeze at –20°C until needed. Extracts should be stored at a temperature compatible with target protein activity; some target proteins may be inactivated by freeze-thaw cycles.

## References

1. Drott, D., Bahairi, S. and Grabski, A. (2002) *in**Nova**tions* **15**, 14–16.
2. (2003) *inNovations* **16**, 22.
3. Okpuzor, J., Seiler, A., Keszenman-Pereyra, D. and Turner, G. (2004) *inNovations* **19**, 15–16.

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